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CLONING AND CHARACTERIZATION OF THE APOPTOTIC SUPPRESSOR GENE P49 FROM SPODOPTERA LITURA NUCLEOPOLYHEDROVIRUS

NORIHA MAT AMIN.

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CLONING AND CHARACTERIZATION OF THE APOPTOTIC SUPPRESSOR GENE p49 FROM *SPODOPTERA LITURA* NUCLEOPOLYHEDROVIRUS

By

NORIHA MAT AMIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CLONING AND CHARACTERIZATION OF THE APOPTOTIC SUPPRESSOR GENE p49 FROM *SPODOPTERA LITURA* NUCLEOPOLYHEDROVIRUS

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September 2005

Chairman: Shuhaimi Mustafa, PhD

Faculty : Biotechnology and Biomolecular Sciences

Baculoviruses possess two types of genes that can suppress apoptosis (programmed cell death), p35 and inhibitor of apoptosis (iap). The p49 gene of a Malaysian isolate of *Spodoptera litura* nucleopolyhedrovirus (SpltMNPV), a homolougue of baculovirus p35 gene was cloned and characterized. The open Reading Frame (ORF) of SpltMNPV p49 gene was 1317 bp long and encodes approximately 349 amino acid residues with a predicted molecular weight of 51.11 kDa. The SpltMNPV p49 gene shared 99% and 87% identity of nucleotide and amino acid sequence respectively to the SpltMNPV (AF325155), SpltMNPV (AF207549) and *Spodoptera littoralis* nucleopolyhedrovirus (SlNPV) p49 (AJ006751) obtained in the GenBank. The SpltMNPV P49 protein showed amino acid identities from 25 to 30% with 37 to 47% similarities to the p35 protein of *Leucania separata*, *Spodoptera litura*, *Rachiplusia ou*, *Autographa californica* and *Bombyx mori* nucleopolyhedroviruses, respectively. The SpltMNPV P49 protein molecule displays a potential caspase recognition site TVTDG at amino acid positions 94 to 98 in the polypeptide chain. The predicted secondary structure of SpltMNPV P49 protein including the

hydrophilic (polar) and hydrophobic (basic) region was found to be similar to other P49 and P35 protein.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGKLONAN DAN PENCIRIAN GEN PENGHALANG APOPTOSIS p49 DARIPADA NUKLEOPOLIHEDROVIRUS YANG MENJANGKITI *SPODOPTERA LITURA*

Oleh

NORIHA MAT AMIN

September 2005

Pengerusi: Shuhaimi Mustafa, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Baculovirus mempunyai dua jenis gen yang boleh menghalang apoptosis (kematian sel yang telah diprogramkan), iaitu p35 dan gen penghalang apoptosis (iap). Gen p49 daripada nukleopolihedrovirus yang menjangkiti larva *Spodoptera litura* di Malaysia (SpltMNPV), satu homolog kepada gen p35 daripada baculovirus telah dipencil dan dicirikan. Gen p49 tersebut didapati mempunyai 1317 pasang bes dan 349 asid amino dengan berat molekul sebanyak 51.11 kilodalton. Gen p49 ini mempunyai 99% persamaan nukleotida dan asid amino dengan gen p49 daripada SpltMNPV (AF325155) dan SpltMNPV (AF207549) manakala 87% persamaan nukleotida dan asid amino didapati apabila dibandingkan dengan gen p49 daripada nukleopolihedrovirus yang menjangkiti larva *Spodoptera littoralis* (SlNPV). Gen p49 ini juga mempunyai persamaan asid amino sekitar 25 hingga 30% dengan 37 hingga 47% kesamaan dengan protein p35 daripada nucleopolihedrovirus yang menjangkiti larva *Leucania separata*, *Spodoptera litura*, *Rachiplusia ou*, *Autographa californica* dan *Bombyx mori*. Molekul protein P49 daripada SpltMNPV mempamerkan satu tapak yang berpotensi sebagai tapak pengenalan enzim caspase pada kedudukan

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asid amino 94 hingga 98 yang diwakili oleh jujukan asid amino TVTDG. Berdasarkan ramalan terhadap struktur sekunder serta kawasan hidrofilik dan hidrofobiknya, protein P49 daripada SpltMNPV di Malaysia didapati mempunyai kesamaan dengan protein P49 dan P35 daripada baculovirus.

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I certify that an Examination Committee has met on 15th September 2005 to conduct the final examination of Noriha Mat Amin on her Master of Science thesis entitled "Cloning and Characterization of the Apoptotic Suppressor Gene p49 from *Spodoptera litura* Nucleopolyhedrovirus" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

MOHD YUNUS ABD SHUKOR, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

MUHAJIR HAMID, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

ABDUL MANAF ALI, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

ISMAIL AHMAD, PhD
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 DEC 2005

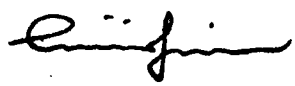
This thesis submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

SHUHAIMI MUSTAFA, PhD
Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

LAU WEI HONG, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

AHMAD SAID SAJAP, PhD
Professor
Faculty of Forestry
Universiti Putra Malaysia
(Member)

HUSSAN ABD KADIR
Malaysian Agricultural Research and Development Institute
MARDI Head Quarters, Serdang
(Member)



AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 JAN 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.

NORIHA MAT AMIN

Date: 20/12/2005

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LIST OF ABBREVIATIONS

A_{260}	absorbance at wavelength 260 nm
A_{600}	absorbance at wavelength 600 nm
AcMNPV	*Autographa californica* multicapsid nucleopolyhedrovirus
Ala (A)	alanine
AnfaNPV	*Anagrapha falcifera* nucleopolyhedrovirus
Arg (R)	arginine
Asn (N)	asparagines
α	alpha
β	beta
bp	base pair
BmNPV	*Bombyx mori* nucleopolyhedrovirus
BV	budded virus
C-terminal	carboxy terminal
Cys (C)	cysteine
dNTPs	deoxyribonucleotide triphosphate mix
DNA	deoxyribonucleic acid
EM	electron microscope
Gln (Q)	glutamine
Glu (E)	glutamic acid
Gly (G)	glycine
h	hour
h.p.i	hour post infection
His (H)	histidine
IAP	inhibitor of apoptosis

Ile (I)	isoleucine
kb	kilobase
kbp	kilobase pair
kDa	kilodalton
kV	kilovolt
LD_{50}	lethal $dose_{50}$
Leu (L)	leucine
LsNPV	*Leucania separata* nucleopolyhedrovirus
Lys (K)	lysine
mM	milimolar
M	molar
MbMNPV	*Mamestra brassicae* multicapsid nucleopolyhedrovirus
NPVs	nucleopolyhedroviruses
N-terminal	amino terminal
OBs	occlusion bodies
ODV	occlusion derived virus
ORF	open reading frames
PCR	polymerase chain reaction
PM	peritrophic membrane
Pro (P)	proline
rpm	rotation per minute
RoMNPV	*Rachiplusia ou* multicapsid nucleopolyhedrovirus
Ser (S)	serine
SlNPV	*Spodoptera littoralis* nucleopolyhedrovirus
SpltMNPV	*Spodoptera litura* multicapsid nucleopolyhedrovirus

SpltNPV	*Spodoptera litura* nucleopolyhedrovirus
TEM	transmission electron microscope
Thr (T)	threonine
Trp (W)	tryptophan
Tyr (Y)	tyrosine
UV	ultraviolet
V	volt
Val (V)	valine
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Spodoptera litura nucleopolyhedrovirus (SpltMNPV) is among the 633 potential baculovirus species and 483 tentative species of NPVs compiled by the ICTV (Murphy *et al.*, 1995; Blissard *et al.*, 2000). SpltMNPV is highly pathogenic to the armyworm, *Spodoptera litura*. Although they are well characterized at the cytological level, there have been only few studies on their DNA characteristics and *in vitro* replication (Maeda *et al.*, 1990) as well as the molecular mechanism of its infection and host specificity (Pang *et al.*, 2001). Several specific SpltMNPV have been reported and characterized (Maeda *et al.*, 1990; Das and Durga prasad, 1996; Hunter-Fujita *et al.*, 1998; Pang *et al.*, 2001; Lau, 2002). It has been tested for controlling *S.litura* larval populations and is recognized as potential alternative for the management of this insect (Takatsuka *et al.*, 2003). Many field trials carried out else where showed that SpltMNPV used either alone or in combination with chemical insecticides could suppress the population of *S. litura* (Su, 1992). In China, India and Taiwan, the formulated product consisting of SpltMNPV has been used against *S. litura* that attacks vegetables, cotton and peanut (Moscardi, 1999) whereas; in the Philippines SpltMNPV alone or in combination with *Bacillus thuriengiensis* effectively controls *S.litura* attacking onion (Lavina *et al.*, 2001).

SpltMNPV variants, which have been isolated from different geographic locations, are found to be slightly diverging between each other when their DNA profile was compared with restriction endonuclease analysis (REN). The

occurrence of genotypic variants of the same virus is common for NPVs, which only differ in the position of a few DNA fragments in the REN profile (Caballero *et al.*, 1992). Because *S.litura* is a highly migratory insect; the distribution of NPV genotypes that infect *S.litura* in the region from which the *S.litura* migrates might be a key determinant for the observed NPV distribution pattern. Other factors including differences in phenotypic characteristics relating to virus fitness such as host range, environmental persistence and the transmission or production of infective units (Takatsuka *et al.*, 2003).

In Malaysia, the pathogenicity of SpltMNPV to *S. litura* has been demonstrated by a few researchers (Kotulai, 1994; Sajap *et al.*, 2000; Lau, 2002). Although the basic properties of SpltMNPV have been characterized (Lau, 2002) informations at the molecular level are still lacking. To date only the polyhedrin gene of SpltMNPV have been cloned and sequenced as well as the size of the whole genome has been determined (Lau, 2002). In this thesis the identification of p49 gene of SpltMNPV, a homologue of p35 gene of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) is presented. p35 is an additional apoptosis-inhibiting gene possessed by some baculoviruses such as AcMNPV. It works in conjunction with an inhibitor of apoptosis genes, IAP (a primary apoptosis-inhibiting gene carried by all baculoviruses) to inhibit apoptosis in a wider range of insect. Because AcMNPV possess this gene, it has broader host range compared to other baculoviruses. On the other hand, *Orgyia pseudotsugata* MNPV (OpMNPV) lacks the p35 gene though it is closely related to AcMNPV thereby showing the narrower host range (Narayanan, 1998). Because this gene plays a role in determining host-range, the fundamental

studies are very important to commercial production of baculoviruses as bioinsecticide. The narrower host range exhibited by some baculoviruses could possibly be improved through insertion of p35-like genes through genetic engineering.

Currently, research is being focused on identifying more p35-like genes in other baculoviruses, as well as other genes that are responsible for controlling species specificity during viral replication. Narrower host range exhibited by baculoviruses usually limits their use as biopesticide because a variety of insects may be infesting a particular crop thus by manipulating these genes host range could be expanded or reduced as desired. Because of the p49 gene found between two closely related NPV species; *Spodoptera littoralis* NPV and *Spodoptera litura* NPV from China showed high sequence homology, it may be possible to search for this gene in the genome of SpltMNPV from Malaysia. Therefore this study was conducted with the following objectives;

1. To isolate p49 gene from the Malaysian *Spodoptera litura* multicapsid nucleopolyhedrovirus (SpltMNPV)
2. To clone and sequence the p49 gene of SpltMNPV
3. To analyze p49 amino acid sequence and compare it with p49 and p35 of other NPV species

CHAPTER 2

LITERATURE REVIEW

2.1 *Spodoptera litura*

2.1.1 Common name

Spodoptera litura (Fabricius), previously known as *Prodenia litura,* is an insect pest species from the order of Lepidoptera and the family of Noctuidae. A very common name for this insect is cluster caterpillar. Others include cotton leafworm, rice cutworm, armyworm and tobacco caterpillar (Hill, 1975; Malaysian Plant Protection Society, 1989; Carter, 1992; Schreiner, 2000).

2.1.2 Host Range

Spodoptera litura is a polyphagous pest of cotton, rice, tomato and tobacco (Hill, 1975). They attack nearly any herbaceous plant including cabbage, cauliflower, beetroot, silverbeet, peanuts, beans, banana, strawberry, apple, lettuce and many other garden plants (Carter, 1992). According to Lavina *et al.* (2001), Okamoto in 1968 have recorded 80 species of host plants including alternate hosts such as weeds and trees. The larvae are primarily leaf feeders but may ocassionally attack young plants at the soil line. They may also feed on the flowers and the green fruit of tomatoes but unlike tomato fruitworms, they generally do not bore into the fruit (Schreiner, 2000).

2.1.3 Distribution

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The moths of this species are widespread throughout the world. It occurs in Australia, Bangladesh, China, Fiji and the Pacific Islands, Hawaii, India, Japan, Korea, Pakistan, Sri Lanka as well as in South East Asia (Hill, 1975; Malaysian Plant Protection Society, 1989; Carter, 1992). This insect is economically important in China, India and Japan, causing considerable economic loss to many vegetable and field crops (Pang *et al.*, 2001). In Malaysia, the occurrence of this pest is sporadic and difficult to predict. The crops can be seriously damaged when heavy infestation occurs. As reported by Sajap *et al.*, 1995, the *Acacia mangium* species at Batu Arang, Selangor and Sungai Sam Forest Reserve in Kelantan have been heavily damaged by this pest.

2.1.4 Life History

The eggs are normally laid in clusters of 200 to 300 underneath leaves and covered with brown scales from the body of the mother. They hatch in three to four days. The newly hatched larvae are tiny, translucent green with a distinct black band on the first abdominal segment (Hill, 1975). The larvae feed in a group when they are young but spread out as they getting older (Schreiner, 2000).

Young caterpillars have smooth skin with a dark patch on the mesothorax. They initially only eat the flesh of their food leaves leaving the veins intact. Later as they grow, they become brownish with three thin yellow lines down the back; one in the middle and one at each side. A row of black dots run along each side and a conspicuous row of dark triangles decorate each side of the back. The last

instars are very dark with four prominent yellow triangles on the mesothorax (Carter, 1992). The virus enters the life cycle of *S. litura* at this stage. Although death usually occurs in the larval stage, some larvae may survive to the pupal or adult stages but the hatchability of the eggs laid by the survived adults may reduced significantly due to the virus infection (Santiago-Alvarez and Vargas-Osuna, 1988).

Pupation normally occurs in the soil below the host plants, several centimeters from the soil surface. The pupa is brownish in color measuring about 18-22mm in length. The duration of the pupal stage lasts about 2-7 days (Mamat and Lim, 1989).

Adults are light to dirty brown with a complex pattern of cream streaks criss-crossing the forewings. It has a wingspan of about 4cm. The hind wings are silvery white. The males have a blue-grey band from the apex to the inner margin of each fore wing (Carter, 1992). The adult moths are nocturnal and their life lasts about 2-8 days, (male, 2-4 days; female, 5-8 days) with mating occuring just 12 hours after emergence and oviposition beginning 12 hours later (Mamat and Lim, 1989).

The whole life cycle takes about 25 days (Schreiner, 2000), 30 days (Hill, 1975; Malaysian Plant Protection Society, 1989) or 13-32 days (Lim and Mamat, 1989). The difference may be attributed to the different food sources provided as well as the conditions under which the insect was reared (Mamat and Lim, 1989).

2.1.5 Control Method

There are various methods available for controlling *Spodoptera litura*. Cultural methods include ploughing and burning of crop stubbles, flooding of infested fields and removal of the weeds (Hill, 1975). In Guam and American Samoa, several parasites have been used to attack the eggs of cluster caterpillar. In the home garden, it is generally possible to simply remove all the small caterpillars in a cluster before much damage to the plant has occurred (Schreiner, 2000).

The application of chemical insecticides to foliage has been widely used. However the chemical control of *S. litura* is ineffective because it has developed resistance to many chemical insecticides. Many cases of resistance of this insect to insecticides have been reported in Japan and India (Hiroshe, 1995; Nagesh Kumar, 1998). The widespread of resistant development is possibly due to the excessive spraying of high doses of chemical pesticides to the crops (Nagesh Kumar, 1998). Several insect pathogens especially the virus, nucleopolyhedrovirus (Shih *et al.*, 1995) and the fungus, *Nomuraea rileyi* (Vimala Devi, 1993) were proved to be useful for supression of this armyworm.

2.2 Baculoviruses

2.2.1 Introduction

The family of Baculoviridae is taxonomically characterized by a large, covalently closed circular double stranded DNA genome, which is packaged in a